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Animal models of anxiety

Anxiety, as an emotional state, is a normal, natural and necessary element of our life. The anxiety usually appears as a distress signal in connection with a definite threat and it modifies the behaviour, reactions, emotions and efficiency of an organism. Reaction of anxiety can have heterogeneous sources and can manifest itself in many ways. Surges of panic are the extreme symptoms of anxiety (1, 13). For a long time, benzodiazepines have been the most popular anxiolytics for treatment of anxiety disorders. These drugs were used for the first time in 1960, and by the end of 1970s they were the favourite drugs in the world. However, all benzodiazepines have many side-effects, including amnesia, ataxia, sedation, rebound anxiety on withdrawal, and development of tolerance in susceptible people (1, 2). Therefore in the psychiatric field there is a great need to create novel therapeutic drugs using new pharmacological targets that provide effective treatments with a rapid clinical onset and few side-effects including antidepressant drugs like selective serotonin inhibitors, 5-HT1a agonists, tricyclic antidepressants and monoamine oxidase inhibitors or beta-blockers (1, 13).

MODELS OF ANXIETY

Information about the way of action of new compounds can lead to new approaches in studying the physiological basis of anxiety. Animal and human anxiety states are viewed as being on a continuum and are based on the similar mechanisms. As such, it is appropriate to examine effects of drugs on the anxiety-related behaviours of animals to predict the potential utility of these drugs in the treatment of human disorders. To demonstrate an anxiolytic effect in animals, it is necessary to create and measure anxiety itself. The aim of the models is to elucidate factors, which may or not be important features of anxiety and the anxiolytic action of the studied drugs. Until now, there are several behavioural models which attempt to involve a number of factors underlying anxiety and fear in humans (9). Models which detect an anxiolytic action are used for two primary reasons: firstly, the screening of new drugs and secondly, the invesitgation of the neurotransmitters involved in anxiety (10). Actually, there is no completely satisfactory animal model of anxiety, but such models are still in use due to a good clinical correlation. A variety of different models may be applied for the investigation of mechanism underlying anxiety and anxiolytic agent's action.

As already mentioned, tests for anxiety in animals (rodents) are useful for assessing the behavioral impact of drugs designed to affect different aspects of anxiety. Moreover, they are very valuable tools in determining the application of genetic factors, which can be used to validate animal models. Except this, they are specifically valuable in identifying the anxiety-related behaviours in these animals, because tests for anxiety in rodents have to allow the animal to display its natural anxiety-related behaviour. Rodents tend to avoid the unprotected area of a new environment, when they first enter it. They will typically start to explore the environment along the walls while avoiding the open i.e. unprotected area. This aversion can be modulated by illumination levels. A brightly lit area can be more aversive for a rat or a mouse than dark one. Another possibility to increase this aversion of rodents against an unprotected area is by elevating

it. When confronted with a threatening stimulus, animals display species-specific behavioural patterns, such as: attends and directed sniffing, which are categorized as risk assessment behaviour and also walking, rearing, climbing, manipulating objects. Such behaviours in rodents are determined by a conflict between the exploration of an unknown area or object and the motivation to avoid a potential danger. Exploration is gradually inhibited by anxiety, thereby representing an indirect measurement of anxiety. Also, exposed to a new environment, rodents are reluctant to eat unknown food. When both familiar and unknown food is presented in a new environment, rodents will typically show a longer reaction to the first intake of unknown food, in comparison with the intake of familiar food (14). However, in rodent tests which measure exploration of a new environment, behavioural effects of the treatments may be contaminated by their intrinsic effects related to locomotor activity (4).

Animal models of anxiety may be subdivided into two basic categories: 1) those that involve unconditioned responses, 2) conditioned avoidance responses (1, 9).

UNCONDITIONED TESTS OF ANXIETY

The most frequently used tests are paradigms for unconditioned anxiety, which are thought to be indicative for human generalized anxiety symptoms. In these tests, rodents usually are confronted with a new environment or stimulus and rodent's behavioural patterns are related to anxiety (14).

OPEN FIELD TEST

One of the earliest and most widely used procedures to asses anxiety-related behaviours is the open field test. It was described for the first time by Hall in 1936, as a test for study emotional states in rats. Hall's apparatus consisted of a brightly-illuminated circular arena of about 1.2 m diameter closed by a wall 0.45 m tall. Rats were placed individually in the outer ring of the open field and their behaviour was observed for 2 min. In the meantime, different types of open fields have been used, varying in terms of shape from circular to square, in terms of illumination from dimly lit to conditions of bright illumination, also in terms of enrichment by offering objects or food, and in size. In a specific variation, also referred to as free exploration test, an open-field is connected to the home cage of the animal which is then permitted a free access to the new environment. In this cage, the number of risk assessment observed in the open field may provide a valuable measure of the approach response toward novelty, that is, exploration (14, 15).

The standard procedure of the open field test implies a forced confrontation with the open area. Briefly, animal is placed either in the center or in the periphery of the area and the following behavioural items are recorded for a period ranging from 2 to 20 min (usually 5 min): horizontal locomotion (number of crossings of the lines marked on the floor), grooming (protracted washing of the coat) or frequency of rearing and leaning (sometimes termed vertical activity) (15). In the open field, the behaviour observed is avoidance of threatening places, which can also be observed in humans. As an indicator for anxiety, the avoidance behaviour towards the unprotected area and the other behavioural patterns related to anxiety are measured (14). An increase in central locomotion or in time spent in the central part of the device without modification of total locomotion and of vertical exploration can be interpreted as an anxiolytic-like effect, while on the contrary, a decrease of these variables is associated with anxiogenic effects (15).

The open field is one of the most popular models of anxiety-like behaviours. It has become so popular that its use has been extended to a great number of species, including lambs, pigs, calves, primates, bush babies, pullets, rabbits or honeybees and lobsters. In fact, it has become a convenient procedure to measure not only anxiety-like behaviours, but also hyperactivity or sedation. The effects of many different drugs have been investigated in the open field, including compounds with effective or potential anxiolytic effects (benzodiazepines, serotonin ligands, neuropeptides), but also compounds with sedative-(neuroleptic), stimulant-(amphetamine and

cocaine), or prostration-inducing (epileptogenic drugs) activity. Although there is an evidence that the open field may be useful in detecting genetic or pharmacological effects on anxiety, some studies also report a lack of sensitivity for anxiety-modulations in this test. It may be a rodent model of normal anxiety, sensitive to the anxiolytic-like effects of classical benzodiazepines and 5-HT1a receptor agonists, but not to the effects in the clinical entity termed "anxiety disorders". The open field test cannot claim predictive validity for anxiety in general, as it is not sensitive to compounds like alprazolam or chronic selective serotonin reuptake inhibitors effective in anxiety disorders, such as social phobias, panics, obsessional compulsive disorders or post-traumatic stress disorders (15).

ELEVATED PLUS MAZE TEST (EPM)

Probably the most frequently used test for unconditioned anxiety is the EPM test, which was introduced for the first time in 1985 (14). The EPM test is a behavioural model of anxiety in rodent, provoked by the novelty and repulsion caused by an elevated and illuminated plus maze (5). The EPM consisted of two open (35x12 cm), two closed arms (35x12x40) and a centre square (12x12 cm). The maze is elevated 50 cm above the floor. Open arms are surrounded by a 0.5 cm ledge and entire floor is covered in black rubber (11). The animal usually is placed in the centre of the EPM, where the four arms cross each other, facing a closed arm and it is allowed to explore the maze for 5 min. The test is carried out in a calm, tempered and dark room. The rodent may explore "unsecured" open arms and/or "secured" closed arms. This paradigm is based on the observation that rodents tend to avoid elevated, open arms, showing a preference for protected arms (closed). Following the concept of avoidance behaviour in rodents, avoidance of the open arms is interpreted as anxiety (14). The anxiolytics effectiveness of a drug is illustrated by a significant augmentation of parameters in open arms (time and/or entries). The augmentation of the percentage of entries in open arms in proportion to total entries in both arms is a good indicator of anxiolysis, although entries in closed arms and total entries reflect the motor component of the exploratory activity (5, 6, 11).

Another version of the EPM test is the unstable elevated exposed plus-maze test (UEEMP). The UEEMP consisted of a plus-shaped maze, which is elevated 50 cm above the floor with four exposed arms and a centre square (12x12 cm). The floor of the maze is covered in black rubber and each arm has a 0.5 cm ledge to prevent slippage. The apparatus is oscillated in the horizontal plane (85 rpm) with a movement amplitude of 2 cm each side of the central point. Rodents are placed on the centre square facing one of the arms 3 sec prior to oscillation beginning (12).

The EPM test has widespread appeal because it is quick and simple. Besides, the equipment of the EPM test is inexpensive and in some laboratories this test is able to detect putative anxiolytics, such as cholecystokinin receptor agonists (CCKb), which lack robust effects in classical animal models of anxiety, i.e. those based on aversive conditioning (6). This model also shows good predictive validity for typical anxiolytics (9). Moreover, it has been shown that the EPM test also allows controlling locomotor activity, thus representing a reliable test for anxiety-modulating properties of pharmacological compounds. Using more complex approaches to analyze behaviour of rodents on the EPM, one can demonstrate increased reliability and sensitivity to this test (14). However, the predictive value of the test is unclear. Although anxiolytics, such as the benzodiazepine receptor agonist chlorodiazepoxide, produce reliable and reproductive effects, other anxiolytics such as the partial 5-HT1a receptor agonists, do not. This suggests that the use of the EPM as an animal model of anxiety has some limitations (6).

DARK/LIGHT TEST

The test is based on a conflict between the aversion of the brightly lit compartment and the spontaneous exploratory behaviour of rodents in response to mild stressor i.e. new environment and light (3, 4). The apparatus consists of two connected compartments, one of which is small,

black and dim and the second large and bright. The small compartment may be painted black and illuminated by a red dim light (60 W, 4 lx), whereas the large one may be painted white and brightly illuminated (60 W, 400 lx light source) (5). In the original setup, the dark compartment is also smaller than the lit one and both compartments are separated by a partition which contains an opening. Later on, some modifications were made in that the dark compartment and the light compartment were equal in size and connected by a small tunnel (14). The transitions between the compartments and time to explore each of them are interpreted as indicators of anxiety. Transitions have been reported to be an index of activity-exploration because of inhibition developing over time, and the time spent in each compartment has been claimed to be the measure of aversion. As a decrease in the dark/light transitions is likely to be confounded with alterations in general activity, it was suggested that the behavioral expression of decreased anxiety in the dark/light box may be determined by genetically-based spontaneous exploration (3, 14).

The dark/light test (also referred to as black/white box), in comparison with the EPM test widely used, yields much more heterogeneous responses (both in controls and benzodiazepine-treated animals). This paradigm may be useful to predict the anxiolytic- and anxiogenic-like activity of drug in rodents (especially in mice). Some authors have shown that this test is sensitive to benzodiazepines. These drugs may increase the number of visits and/or time spent in brightly lit area, while decreasing the exploration of the dark compartment. In addition, repeated benzodiazepine treatment has been shown to increase the percentage of time spent by mice in the light compartment (4). The newer anxiolytic-like compounds (e.g. serotonergic drugs or drugs acting on neuropeptide receptors) can be also detected using this test. The dark/light test has other advantages of being quick and easy to use, without the prior training of animal. Moreover, food and water deprivation is unnecessary and natural stimuli are used. It is worth mentioning that this test differs from other models of anxiety, which are not equivalent in terms of elicited/induced emotional state (3).

THE MODIFIED HOLE BOARD (mHB)

The hole board is based on the concept that the rich behavioral patterns of rodents can only be displayed in an adequate (i.e. rich) testing environment. The mHB measured 60x60 cm and is surrounded by a wall 35 cm high. Four 2.5 diameter holes, 15 cm from each corner, are located on the floor (11). In the mHB paradigm, the hole board, with all holes being covered by a movable lid, is placed in the middle of a box, thus representing the central area. The experimental box is enlarged by an additional compartment, where the animal testing group is placed during the test period, and is separated from the test area by a transparent partition. It has been shown that in both rats and mice, mHb enables the investigator to detect alterations in a wide range of behaviours, including anxiety-related behaviour, exploration, risk assessment, arousal, social affinity, locomotor activity and different cognitive processes (14). For example, locomotion is assessed by distance moved (cm), rearing (frequency) and reartime (seconds spent on rearing in a trial), but exploration is analyzed by frequency of head dipping into any of the holes (11).

SOCIAL INTERACTION TEST

The social interaction test of anxiety was developed 25 years ago. For the first time, this test used animals as an ethologically relevant source of anxiety. Social interaction test measured natural behaviours, which are related with anxiety. This test was sensitive to both anxiolytic and anxiogenic stimuli. Thus, this paradigm played a crucial role in measurement of anxiety. Social interaction test required neither the deprivation of food and water nor electric shock and did not need extensive training of the animal. The dependent variable is the time spent by pairs of male rats in social interaction (e.g. sniffing, grooming or following the partner). Because the behaviour of one rat influences that of the other, the pair of rats is treated as a unit. An increase in social interaction, without an accompanying increase in motor activity, is indicative of an anxiolytic

effect, whereas a specific decrease in social interaction indicates an anxiogenic effect. There are four test conditions: low light, familiar arena; high light, familiar arena; high light, unfamiliar arena; low light, unfamiliar arena. Social interaction is the highest when rats are tested in a familiar arena lit by low light, and it decreases as the test conditions become more aversive or anxiogenic. Moreover, this test can be used among mice or gerbils. Analysis has shown that both mice and gerbils showed the same decrease in the social interaction as rats when the light level was manipulated, but the changes in response to the familiarity of the test arena were less reliable. The social interaction test of anxiety has proved useful in detecting both anxiolytic and anxiogenic effects of environmental factors and systematically administered drugs. It has been also proved helpful in unraveling the neural basis of anxiety. It is hoped that the next quarter of a century of research using this test will prove as interesting and fruitful as the last, and the test will play a crucial role in determining the genetic basis of anxiety disorders (7).

CONDITIONED TESTS OF ANXIETY

Conditioning models involve the pairing of an unconditioned response with (usually) an aversive stimulus and, therefore, may model reactions to specific aversive events (or stimuli paired with them) (9). Another behavioural approach used to assess different aspects of anxiety in animal relies on conflict tests in combination with punishment, mostly induced by an electric foot shock. Due to ethical considerations on the question of whether or not electric shocks may represent a relevant stimulus, tests which are based on electric shock are less often used than tests for unconditioned anxiety (14).

QUICK GUIDE

In the early 1940s, researches first described a conditioned emotional response paradigm in which a freely moving rat is trained to press a lever for occasional food rewards. During training, a light is switched on for a short time, usually 60 sec. This light signals the delivery of a mild electric shock. After a number of light-shock, the rat presses the lever more seldom when the light is on, because it assumes anxious imminent delivery of the shock (6).

FOUR PLATES TEST

The apparatus consists of a cage with a floor composed of four metal plates connected to a device that can generate electric shocks (0.6 mA, 0.5 s). The top of the cage is covered by a transparent Perspex lid which prevents escape of an animal. Following a 15 s latency period, the rodent is subjected to an electric shock after crossing from one plate to another. The number of crossing is measured during a 1 min test period (5).

THE VOGEL CONFLICT TEST

This paradigm has been modified from the Geller-Seifter test, in which the initially-used food reward was replaced by a water reward. The Vogel conflict test is based on a conditioning procedure in which water-deprived animals are given access to a bottle of water during the test situation. Randomly, a lick by a rodent is accompanied by an electric shock. Anxiolytic effects are indicated by modulation of the shock-induced suppression of licking (14).

Although there are many variations of conditioned test in general, some anxiolytics (such as benzodiazepines) yield reliable and reproductive results regardless of the details of the procedure. In any case, they may reflect different aspects of anxiety. In addition, the same animals can be used a number of times, and comparison with standard compounds can be made within the same experimental group. However, these models have also several disadvantages. Firstly, some anxiolytics such as CCKb or 5-HT3 receptor agonists do not have strong effects in conditioned

anxiety models. Moreover, as an anxiolytic effect is usually indicated by an increase in a specific behaviour such as lever pressing for food, sedative/muscle relaxant effects can confound results because they reduce the animal's capacity to perform the task. Finally, the animals should be deprived of food or water during the experiments, therefore compounds that affect motivation for these rewards can lead to false positives or false negatives results (6). Notably, the results of the tests can be strongly influenced by testing conditions and the specific test procedure used, and it is necessary to define carefully these factors when testing for anxiety in animals (14).

CONCLUSIONS

Recently, there has been an increase in the number of tests proposed to be animal models of anxiety, and it is too early to evaluate many of them (10). Pre-clinical models of anxiety have failed to provide consistent profiles for establishing novel anxiolytic agents (11). It is essential to relate at least one animal test to each anxiety disorder, i.e. general anxiety, phobia, panic disorder or obsessive compulsive disorder and post-traumatic stress (10). In the table below, both advantages and disadvantages of unconditioned and conditioned models of anxiety have been summarized (6).

Table 1. Advantages and disadvantages of animal models of anxiety

	Advantages	Disadvantages
Unconditioned (ethological) model	often quick and easy to use, equipment is inexpensive, training of animals is not required, food or water deprivation is not required, natural stimuli are used.	anxiolytic and anxiogenic effects are confounded by changes in motor activity, baseline measures are subject to marked day-to-day variation, animal cannot be reused, effects of chronic drug administration cannot be investigated because exploratory behaviour habituates, effects often cannot be reproduced within and between laboratories.
Conditioned model	baseline measures are consistent and reproducible within and between laboratories, animals can be reused, good predictor of a drug's anxiolytic potential in humans.	animals have to undergo long training periods, food or water deprivation is required, only benzodiazepines have consistent effects, sedation and muscle relaxation can affect the animal's ability to perform the behaviour.

REFERENCES

1. Belzung C., Griebel G.: Measuring normal and pathological anxiety-like behavior in mice: review. Behav. Brain Res., 125, 141, 2001.
2. Bonn D., Bonn J.: Anxious times for the treatment of anxiety. The Lancet, 352, 1126, 1998.
3. Bourin M., Hascoët M.: The mouse light/dark box test. Eur. J. Pharmacol., 463, 55, 2003.
4. Chaouloff F. et al.: Anxiety- and activity-related effects of diazepam and chlorodiazepoxide in the rat light/dark and dark/light tests. Behav. Brain Res., 85, 27, 1997.
5. Clenet F. et al.: Anxiolytic profile of HG1, a 5-HT-moduline antagonist, in three mouse models of anxiety. Eur. Neuropsychopharmacol.,14, 449, 2004.

6. Dawson G.R., Tricklebank M.D.: Use of the elevated plus maze in the search for novel anxiolytic agents. Trends Pharmacol. Sci., 16, 2, 33, 1995.
7. File S., Seth P.: A review of 25 years of the social interaction test. Eur. J. Pharmacol., 463, 35, 2003.
8. Flint J.: Animal models of anxiety and their molecular dissection. Seminars in Cell & Devel. Biology, 14, 37, 2003.
9. Hendrie C.A. et al.: Exploration and predation models of anxiety: evidence from laboratory and wild species. Pharmacol. Biochem. Behav., 54, 1, 13, 1996.
10. Howard J.L., Pollard C.T.: Effects of drug on punished behavior: Preclinical test for anxiolytics. In: Psychopharmacology of Anxiolytics and Antidepressants, 131, 1991.
11. Jones N., King S.M.: Influence of circadian phase and test illumination on pre-clinical models of anxiety. Physiol. Behav., 72, 99, 2001.
12. King S.M.: Escape-related behaviors in an unstable elevated and exposed environment. Behav. Brain Res., 98, 113, 1999.
13. Marks I.M., Nesse R.M.: Fear and fitness: An evolutionary analysis of anxiety disorders. Ethol. Sociobiol., 15, 247, 1994.
14. Ohl F.: Testing for anxiety. Clinic. Neurosci. Res., 3, 233, 2003.
15. Prut L., Belzung C.: The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. Eur. J. Pharmacol., 463, 3, 2003.

SUMMARY

Human anxiety disorders are a major world health problem. In the search for new anxiolytics, drugs' companies are systematically looking for the best animal model of anxiety. The assessment of anxiety-related behaviour in animal models is based on the assumption that anxiety in animals is comparable to anxiety in humans. Different models using to express or measure anxiety in animals are based on spontaneous behaviors (exploration, food intake) or on conditioned anxiety. This paper describes test paradigms of both conditioned and unconditioned anxiety, demonstrates the importance of ethological and evolutionary considerations, which can enhance the utility and relevance of various animal models of anxiety. The purpose of the present paper is an attempt to describe a review of almost all animal tests in order to indicate the best one possible.

Zwierzęce modele lęku

W ostatnich latach lęk staje się powszechnym problemem zdrowotnym populacji ludzkiej, dlatego niezbędne jest poszukiwanie nowych leków przeciwlękowych o jak najmniejszych skutkach ubocznych, a szerszym spektrum działania. Do poszukiwania i testowania takich leków konieczne jest posiadanie odpowiedniego modelu zwierzęcego. W pracy przedstawiono najważniejsze zwierzęce modele lęku i starano się ocenić stopień wykorzystania tych modeli w odniesieniu do skuteczności terapii przeciwlękowej.